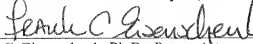


I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on January 10, 2011.

  
Frank C. Eisenschenk, Ph.D., Patent Attorney

REQUEST FOR CERTIFICATE OF  
CORRECTION UNDER 37 CFR 1.322  
AND UNDER 37 CFR 1.323  
Docket No. SER.111  
Patent No. 7,846,427

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Maria Dorly Del Curto  
Issued : December 7, 2010  
Patent No. : 7,846,427  
Conf. No. : 1354  
For : Stabilized Interferon Liquid Formulations

Mail Stop Certificate of Corrections Branch  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

REQUEST FOR CERTIFICATE OF CORRECTION  
UNDER 37 CFR 1.322 (OFFICE MISTAKE) AND  
UNDER 37 CFR 1.323 (APPLICANT MISTAKE)

Sir:

A Certificate of Correction for the above-identified patent has been prepared and is attached hereto.

In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

**Patent Reads:**

Column 1, line 60:

“Burkit’s lymphoma”

Column 2, line 10:

“There is a duster”

**Application Reads:**

Page 2, line 2:

--Burkitt’s lymphoma--

Page 2, line 15:

--There is a cluster--

**Patent Reads:**Column 2, line 19:

“agents can are sometimes”

Column 2, lines 29-30:

“166 amino acid”

**Patent Reads:**Column 3, line 4:

“cyclodextrins are”

Column 3, line 18:

“(2,6-dimethyl)-beta-cyclodextrin”

**Patent Reads:**Column 3, line 31:

“has bee”

**Patent Reads:**Column 3, line 32:

“T. Ide”

Column 3, line 40:

“stabilization if”

**Patent Reads:**Column 4, line 2:

“hum an serum”

**Application Should Read:**Page 2, line 20:

--agents are sometimes--

Page 2, line 27:

--166 amino acids--

**Application Reads:**Page 3, line 25:

--cyclodextrins are--

Page 4, line 2:

--(2,6-di-O-methyl)-beta-cyclodextrin--

**Application Should Read:**Page 4, line 13:

--has been--

**Application Reads:**Page 4, line 13:

--T. Irie--

Page 4, line 18:

--stabilization of--

**Application Should Read:**Page 5, line 4:

--human serum--

**Patent Reads:**Column 4, line 58:

“polynucleotide’s”

Column 5, line 7:

“amino adds”

Column 5, line 14:

“amino add”

Column 7, line 65:

“sulfuric add, and salts with organic adds”

Column 7, line 66:

“oxalic add”

Column 8, line 7:

“so called”

Column 8, line 23:

“amino add”

Column 8, line 32:

“tripe tide”

Column 8, line 43:“an Ig<sub>2</sub>, molecule.”**Patent Reads:**Column 8, lines 64-65:

“80 000 IU/kg and 200 000”

**Application Reads:**Page 6, line 12:

--polynucleotides--

Page 6, line 23:

--amino acids--

Page 6, line 28:

--amino acid--

Page 10, lines 28-29:

--sulfuric acid, and salts with organic acids--

Page 10, line 29:

--oxalic acid--

Page 11, line 1:

--so-called--

Page 11, line 12:

--amino acid--

Page 11, line 19:

--tripeptide--

Page 11, lines 25-26:

--an Ig molecule.--

**Application Should Read:**Page 12, line 8:

--80,000 IU/kg and 200,000--

Column 9, line 9:

“one, twice”

**Patent Reads:**

Column 9, line 15:

“IFN- $\beta$  In the”

Column 10, lines 42-43:

“hydroxypropyl-gamma-cyclodextrin”

Column 10, line 45:

“or GIBCD)”

Column 10, line 50:

“maltotriosylgamma-cyclodextrin”

Column 10, lines 52-53:

“maltosyl-beta-cydodextrin/dimaltosyl-beta-dodextrin”

Column 11, line 21:

“bacterostatic”

Column 11, line 26:

“product Examples”

Column 13, line 4:

“IFN beta”

Column 13, line 14:

“in the are buffer”

Page 12, line 17:

--once, twice--

**Application Reads:**

Page 12, line 22:

--IFN- $\beta$  in the--

Page 14, line 22:

--hydroxypropyl-gamma-cyclodextrin--

Page 14, line 24:

--or G<sub>I</sub>BCD)--

Page 14, line 27:

--maltotriosyl-gamma-cyclodextrin--

Page 14, line 29:

--maltosyl-beta-cyclodextrin/dimaltosyl-beta-cyclodextrin--

Page 15, line 23:

--bacteriostatic--

Page 15, lines 26-27:

--product. Examples--

Page 18, line 8:

--IFN-beta--

Page 18, line 15:

--in the art. Buffer--

**Patent Reads:**Column 15, line 40:

“the invention include”

**Patent Reads:**Column 16, line 53:

“amount if”

Column 16, line 65:

“amount if”

Column 17, line 7:

“malting”

Column 17, line 46:

“and 5(0x M”

Column 18, line 48:

“5 p”

Column 18, line 59:

“up to 10 µg”

Column 19, line 5:

“Hydroxypropyl-betacyclodextrin”

Column 19, line 56:

“isotonicity”

Column 20, line 26:

“manner at”

**Application Should Read:**Page 22, line 3:

--the invention includes--

**Application Reads:**Page 23, line 28:

--amount of--

Page 24, line 5:

--amount of--

Page 24, line 12:

--melting--

Page 25, line 12:

--and 500x M--

Page 27, line 2:

--5 µ--

Page 27, line 13:

--up to 100µg--

Page 27, line 24:

--Hydroxypropyl-beta-cyclodextrin--

Page 28, line 26:

--isotonicity--

Page 29, line 20:

--manner: at--

Column 20, line 31:

“excipient interferon”

**Patent Reads:**

Column 23, line 12:

“about 4-0”

**Patent Reads:**

Column 23, line 14:

“diluted 12”

Column 23, line 21:

“(OD)<sub>380 nm</sub>”

Column 23, line 36:

“OD 96 nm”

Column 24, line 11:

“a-helix”

Column 25, line 8:

“8. T. Irie”

Column 25, line 59:

“140 (1987)”

Column 25, line 64:

“22. Wang et al.”

Page 29, line 24:

--excipient/interferon--

**Application Should Read:**

Page 34, line 10:

--about 4.0--

**Application Reads:**

Page 34, line 12:

--diluted 1:2--

Page 34, line 17:

--(OD)<sub>360nm</sub>--

Page 34, line 27:

--OD<sub>360 nm</sub>--

Page 35, line 24:

-- $\alpha$ -helix--

Page 37, line 15:

--8. Irie--

Page 37, line 33:

--1-40 (1987)--

Page 38, line 5:

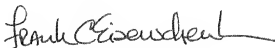
--22. Wang et al.--

A true and correct copy of pages 2-4, 6, 10-12, 14, 15, 18, 23-25, 27-29, 34, 35, 37 and 38 of the specification as filed which support Applicants' assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

The fee of \$100.00 was paid at the time this Request was filed. The Commissioner is also authorized to charge any additional fees as required under 37 C.F.R. 1.20(a) to Deposit Account No. 19-0065.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



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FCE/jb

Attachments: Copy of pages 2-4, 6, 10-12, 14, 15, 18, 23-25, 27-29, 34, 35, 37 and 38 of the specification

IFN is IFN- $\gamma$ . There is also a fourth type of IFN, lymphoblastoid IFN, produced in the "Namalwa" cell line (derived from Burkitt's lymphoma), which seems to produce a mixture of both leukocyte and fibroblast IFN.

- The interferon unit or International unit for interferon (U or IU, for international unit) has been reported as a measure of IFN activity defined as the amount necessary to protect 50% of the cells against viral damage. The assay that may be used to measure bioactivity is the cytopathic effect inhibition assay as described (*Rubinstein, et al. 1981; Familletti, P. C., et al., 1981*). In this antiviral assay for interferon about 1 unit/ml of interferon is the quantity necessary to produce a cytopathic effect of 50%.
- The units are determined with respect to the International reference standard for Hu-IFN-beta provided by the National Institutes of Health (*Pestka, S. 1986*).

Every class of IFN contains several distinct types. IFN- $\beta$  and IFN- $\gamma$  are each the product of a single gene.

- The proteins classified as IFNs- $\alpha$  are the most diverse group, containing about 15 types. There is a cluster of IFN- $\alpha$  genes on chromosome 9, containing at least 23 members, of which 15 are active and transcribed. Mature IFNs- $\alpha$  are not glycosylated.

- IFNs- $\alpha$  and IFN- $\beta$  are all the same length (165 or 166 amino acids) with similar biological activities. IFNs- $\gamma$  are 146 amino acids in length, and resemble the  $\alpha$  and  $\beta$  classes less closely. Only IFNs- $\gamma$  can activate macrophages or induce the maturation of killer T cells. These new types of therapeutic agents can are sometimes called biologic response modifiers (BRMs), because they have an effect on the response of the organism to the tumor, affecting recognition via immunomodulation.

- Human fibroblast Interferon (IFN- $\beta$ ) has antiviral activity and can also stimulate natural killer cells against neoplastic cells. It is a polypeptide of about 20,000 Da induced by viruses and double-stranded RNAs. From the nucleotide sequence of the gene for fibroblast Interferon, cloned by recombinant DNA technology, (*Derynk et al. 1980*) deduced the complete amino acid sequence of the protein. It is 166 amino acid long.

- Shepard et al.* (1981) described a mutation at base 842 (Cys  $\rightarrow$  Tyr at position 141) that abolished its anti-viral activity, and a variant clone with a deletion of nucleotides 1119-1121.

*Mark et al.* (1984) inserted an artificial mutation by replacing base 469 (T) with (A) causing an amino acid switch from Cys  $\rightarrow$  Ser at position 17. The resulting IFN- $\beta$

was reported to be as active as the 'native' IFN- $\beta$  and stable during long-term storage (-70°C).

Rebif® (Serono - recombinant human interferon- $\beta$ ), the latest development in interferon therapy for multiple sclerosis (MS), is interferon(IFN)-beta-1a, produced from mammalian cell lines. Its recommended International Non-proprietary Name (INN) is "Interferon beta-1a".

As with all protein-based pharmaceuticals, one major obstacle that must be overcome in the use of IFN-beta as a therapeutic agent, is the loss of pharmaceutical efficacy that can result from its instability in pharmaceutical formulations.

Physical Instabilities that threaten polypeptide activity and efficacy in pharmaceutical formulations include denaturation and formation of soluble and insoluble aggregates, while chemical instabilities include hydrolysis, imide formation, oxidation, racemization, and deamidation. Some of these changes are known to lead to the loss or reduction of the pharmaceutical activity of the protein of interest. In other cases, the precise effects of these changes are unknown, but the resulting degradative products are still considered to be pharmaceutically unacceptable due to the potential for undesirable side effects.

The stabilization of polypeptides in pharmaceutical compositions remains an area in which trial and error plays a major role (reviewed by Wang (1999) *Int. J. Pharm.* 185:129-188; Wang and Hanson (1988) *J. Parenteral Sci. Tech.* 42:S3-S26). Excipients that are added to polypeptide pharmaceutical formulations to increase their stability include buffers, sugars, surfactants, amino acids, polyethylene glycols, and polymers, but the stabilizing effects of these chemical additives vary depending on the protein.

Cyclodextrins are cyclic oligosaccharides. The most common cyclodextrins are alpha-cyclodextrin, which is composed of a ring of six glucose residues; beta-cyclodextrin, which is composed of a ring of seven glucose residues; and gamma-cyclodextrin, which is composed of a ring of eight glucose units. The inside cavity of a cyclodextrin is lipophilic, while the outside of the cyclodextrin is hydrophilic; this combination of properties has led to widespread study of the cyclodextrins, particularly in connection with pharmaceuticals, and many inclusion complexes have been reported. Beta-cyclodextrin has been of special interest because of its cavity size, but its relatively low aqueous solubility (about 1.8% w/v at 25°C) and attendant nephrotoxicity have limited its use in the pharmaceutical field.

- Attempts to modify the properties of the natural cyclodextrins have resulted in the development of heptakis (2,6-di-O-methyl)-beta-cyclodextrin, heptakis (2,3,6-tri-O-methyl)-beta-cyclodextrin, hydroxypropyl-beta-cyclodextrin, beta-cyclodextrin-epichlorohydrin polymer and others. For a comprehensive review of cyclodextrins and their use in pharmaceutical research, see *Pitha et al, in Controlled Drug Delivery*, ed. S. D. Bruck, Vol. 1, CRC Press, Boca Raton, Fla., pp. 125-148 (1983). For an even more recent overview, see *Uekama et al, in CRC Critical Reviews in Therapeutic Drug Carrier Systems*, Vol. 3 (1), 1-40 (1987); *Uekama, in Topics in Pharmaceutical Sciences 1987*, eds. D. D. Breimer and P. Spelsers, Elsevier Science Publishers B.V. (Biomedical Division), 181-194 (1987); and *Pagington, Chemistry in Britain*, pp. 455-458 (May 1987). The use of cyclodextrins specifically in the field of peptide and protein delivery has been reviewed by *T. Irie et al in Adv. Drug Deliv. Rev.*, Vol 36, 101-123 (1999) and examples in the case of ovine growth hormone, interleukin-2 and bovine insulin are described in *Brewster et al., 1991, Pharmaceutical Research*, 8(6), 792-795.

WO 90/03784 and US 5,997,856 describe a method for the solubilization and/or stabilization of polypeptides, especially proteins, by means of cyclodextrins. However no data on the stabilization of interferons are reported in this document.

- US 6,582,728 describes dry powder compositions for pulmonary administration containing Interferon-beta also containing human serum albumin, which can further contain cyclodextrins. However, even in this case no data on the stabilization of Interferon composition containing also cyclodextrins are reported in this document.

WO 2003/002162 described stabilized compositions comprising an interferon molecule and a specific derivative of cyclodextrin, i.e. sulfoalkyl ether cyclodextrin.

- Consequently, there is a need for additional IFN-beta pharmaceutical compositions comprising physiologically compatible stabilizers that improve the solubility of this protein and stabilize the protein against aggregate formation, thereby enhancing their pharmaceutical utility.

#### 30 DESCRIPTION OF THE INVENTION

The present invention is directed to stabilized pharmaceutical compositions that comprise an Interferon (IFN), methods for their preparation and use thereof. In particular the main object of the invention is to provide a stabilized liquid pharmaceutical composition comprising an Interferon (IFN) or an isoform, mutein, fused

Any such mutein preferably has a sequence of amino acids sufficiently duplicative of that of IFN, such as to have substantially similar or even better activity to an IFN. The biological function of interferon is well known to the person skilled in the art, and biological standards are established and available e.g. from the National  
5 Institute for Biological Standards and Control (<http://immunology.org/links/NIBSC>).

Bioassays for the determination of IFN activity have been described. An IFN assay may for example be carried out as described by Rubinstein et al., 1981. Thus, it can be determined whether any given mutein has substantially a similar, or even a better, activity than IFN by means of routine experimentation.

10 Muteins of IFN, which can be used in accordance with the present invention, or nucleic acid coding therefore, include a finite set of substantially corresponding sequences as substitution peptides or polynucleotides which can be routinely obtained by one of ordinary skill in the art, without undue experimentation, based on the teachings and guidance presented herein.

15 Preferred changes for muteins in accordance with the present invention are what are known as "conservative" substitutions. Conservative amino acid substitutions of polypeptides or proteins of the invention, may include synonymous amino acids within a group, which have sufficiently similar physicochemical properties that substitution between members of the group will preserve the biological function of the  
20 molecule. It is clear that insertions and deletions of amino acids may also be made in the above-defined sequences without altering their function, particularly if the insertions or deletions only involve a few amino acids, e.g., under thirty, and preferably under ten, and do not remove or displace amino acids which are critical to a functional conformation, e.g., cysteine residues. Proteins and muteins produced by such  
25 deletions and/or insertions come within the purview of the present invention.

Preferably, the synonymous amino acid groups are those defined in Table I. More preferably, the synonymous amino acid groups are those defined in Table II; and most preferably the synonymous amino acid groups are those defined in Table III.

fluids. An IFN may thus be fused to another protein, polypeptide or the like, *e.g.*, an immunoglobulin or a fragment thereof.

"Functional derivatives" as used herein cover derivatives of IFN, and their mutants and fused proteins, which may be prepared from the functional groups which occur as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, *i.e.* they do not destroy the activity of the protein which is substantially similar to the activity IFN, and do not confer toxic properties on compositions containing it. These derivatives may, for example, include polyethylene glycol side-chains, which may mask antigenic sites and extend the residence of IFN in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (*e.g.* alkanoyl or carbocyclic aryl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl or threonyl residues) formed with acyl moieties.

As "active fractions" of IFN, or mutants and fused proteins, the present invention covers any fragment or precursors of the polypeptide chain of the protein molecule alone or together with associated molecules or residues linked thereto, *e.g.*, sugar or phosphate residues, or aggregates of the protein molecule or the sugar residues by themselves, provided said fraction has no significantly reduced activity as compared to the corresponding IFN.

The term "salts" herein refers to both salts of carboxyl groups and to acid addition salts of amino groups of the proteins described above or analogs thereof. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases as those formed, for example, with amines, such as triethanolamine, arginine or lysine, piperidine, procaine and the like. Acid addition salts include, for example, salts with mineral acids, such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids, such as, for example, acetic acid or oxalic acid. Of course, any such salts must retain the biological activity of the proteins (IFN) relevant to the present invention, *i.e.*, the ability to bind to the corresponding receptor and initiate receptor signalling.

In accordance with the present invention, the use of recombinant human IFN-beta and the compounds of the invention is further particularly preferred.

A special kind of interferon variant has been described recently. The so-called "consensus interferons" are non-naturally occurring variants of IFN (US 6,013,253). According to a preferred embodiment of the invention, the compounds of the invention are used in combination with a consensus interferon.

5 As used herein, human interferon consensus (IFN-con) shall mean a non-naturally-occurring polypeptide, which predominantly includes those amino acid residues that are common to a subset of IFN- $\alpha$ 's representative of the majority of the naturally-occurring human leukocyte interferon subtype sequences and which includes, at one or more of those positions where there is no amino acid common to all  
10 subtypes, an amino acid which predominantly occurs at that position and in no event includes any amino acid residue which is not existent in that position in at least one naturally-occurring subtype. IFN-con encompasses but is not limited to the amino acid sequences designated IFN-con1, IFN-con2 and IFN-con3 which are disclosed in U.S. 4,695,623, 4,897,471 and 5,541,293. DNA sequences encoding IFN-con may be  
15 produced as described in the above-mentioned patents, or by other standard methods.

In a further preferred embodiment, the fused protein comprises an Ig fusion. The fusion may be direct, or via a short linker peptide which can be as short as 1 to 3 amino acid residues in length or longer, for example, 13 amino acid residues in length. Said linker may be a tripeptide of the sequence E-F-M (Glu-Phe-Met) (SEQ ID:1), for  
20 example, or a 13-amino acid linker sequence comprising Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met (SEQ ID:2) introduced between the sequence of IFN and the immunoglobulin sequence. The resulting fusion protein may have improved properties, such as an extended residence time in body fluids (half-life), increased specific activity, increased expression level, or the purification of the fusion protein is facilitated.

25 In a further preferred embodiment, IFN is fused to the constant region of an Ig molecule. Preferably, it is fused to heavy chain regions, like the CH<sub>2</sub> and CH<sub>3</sub> domains of human IgG1, for example. Other isoforms of Ig molecules are also suitable for the generation of fusion proteins according to the present invention, such as isoforms IgG<sub>2</sub>, IgG<sub>3</sub> or IgG<sub>4</sub>, or other Ig classes, like IgM or IgA, for example. Fusion proteins may be  
30 monomeric or multimeric, hetero- or homomultimeric.

In a further preferred embodiment, the functional derivative comprises at least one moiety attached to one or more functional groups, which occur as one or more side chains on the amino acid residues. Preferably, the moiety is a polyethylene (PEG)

moiety. PEGylation may be carried out by known methods, such as the ones described in WO 99/55377, for example.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Standard dosages of human IFN-beta range from 80 000 IU/kg and 200 000 IU/kg per day or 6 MIU (million international units) and 12 MIU per person per day or 22 to 44 µg (microgram) per person. In accordance with the present invention, IFN may preferably be administered at a dosage of about 1 to 50 µg, more preferably of about 10 to 30 µg or about 10 to 20 µg per person per day.

The administration of active ingredients in accordance with the present invention may be by intravenous, intramuscular or subcutaneous route. The preferred route of administration for IFN is the subcutaneous route.

IFN may also be administered daily or every other day, or less frequently. Preferably, IFN is administered one, twice or three times per week.

The preferred route of administration is subcutaneous administration, administered e.g. three times a week. A further preferred route of administration is the intramuscular administration, which may e.g. be applied once a week.

The dosing of IFN-β in the treatment of relapsing-remitting MS according to the invention depends on the type of IFN-β used.

In accordance with the present invention, where IFN is recombinant IFN-β1b produced in E. Coli, commercially available under the trademark Betaseron®, it may preferably be administered sub-cutaneously every second day at a dosage of about of 250 to 300 µg or 8 MIU to 9.6 MIU per person.

In accordance with the present invention, where IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Avonex®, it may preferably be administered intra-muscularly once a week at a dosage of about of 30 µg to 33 µg or 6 MIU to 6.6 MIU per person.

In accordance with the present invention, when IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under

period of at least at or about 12 hours, 24 hours, 48 hours, etc., preferably up to a period of at or about 12 days. The injections may be spaced in time, for example, by a period of 6, 12, 24, 48 or 72 hours.

- The term "buffer" or "physiologically-acceptable buffer" refers to solutions of compounds that are known to be safe for pharmaceutical or veterinary use in formulations and that have the effect of maintaining or controlling the pH of the formulation in the pH range desired for the formulation. Acceptable buffers for controlling pH at a moderately acidic pH to a moderately basic pH include, but are not limited to, such compounds as phosphate, acetate, citrate, arginine, TRIS, and histidine. "TRIS" refers to 2-amino-2-hydroxymethyl-1,3,-propanediol, and to any pharmacologically acceptable salt thereof. Preferable buffers are acetate buffers with saline or an acceptable salt.

- The "cyclodextrins" contemplated for use herein are hydroxypropyl, hydroxyethyl, glucosyl, maltosyl and maltotriosyl derivatives of beta-cyclodextrin and the corresponding derivatives of gamma-cyclodextrin. The hydroxyalkyl groupings may contain one or more hydroxyl groups, e.g. hydroxypropyl (2-hydroxypropyl, 3-hydroxypropyl), dihydroxypropyl and the like. The glucosyl, maltosyl and maltotriosyl derivatives may contain one or more sugar residues, e.g. glucosyl or diglucosyl, maltosyl or dimaltosyl. Various mixtures of the cyclodextrin derivatives may be used as well, e.g. a mixture of maltosyl and dimaltosyl derivatives. Specific cyclodextrin derivatives for use herein include hydroxypropyl-beta-cyclodextrin (HPCD or HPBCD), hydroxyethyl-beta-cyclodextrin (HEBCD), hydroxypropyl-gamma-cyclodextrin (HPGCD), hydroxyethyl-gamma-cyclodextrin (HEGCD), dihydroxypropyl-beta-cyclodextrin (2HPBCD), glucosyl-beta-cyclodextrin (G<sub>1</sub>-beta-CD or G<sub>1</sub>BCD), diglucosyl-beta-cyclodextrin (2G G<sub>1</sub>-beta-CD or 2 G<sub>1</sub>BCD), maltosyl-beta-cyclodextrin (G<sub>2</sub>-beta-CD or G<sub>2</sub>BCD), maltosyl-gamma-cyclodextrin (G<sub>2</sub>-gamma-CD or G<sub>2</sub>GCD), maltotriosyl-beta-cyclodextrin (G<sub>3</sub>-beta-CD or G<sub>3</sub>BCD), maltotriosyl-gamma-cyclodextrin (G<sub>3</sub>-gamma-CD or G<sub>3</sub>GCD) and dimaltosyl-beta-cyclodextrin (2 G<sub>2</sub>-beta-CD or 2 G<sub>2</sub>BCD), and mixtures thereof such as maltosyl-beta-cyclodextrin/dimaltosyl-beta-cyclodextrin.

- Hydroxypropyl-beta-cyclodextrin for use in the compositions of the present invention is commercially available and is the preferred cyclodextrin according to the invention.

Alternatively, it may be prepared by known methods, especially by use of the optimized procedure of *Pitha et al*, *International Journal of Pharmaceutics*, 29, 73-82 (1986).

5 An "isotonicity agent" is a compound that is physiologically tolerated and imparts a suitable tonicity to a formulation to prevent the net flow of water across cell membranes that are in contact with the formulation. Compounds such as glycerin, are commonly used for such purposes at known concentrations. Other suitable isotonicity agents include, but are not limited to, amino acids or proteins (e.g., glycine or albumin), salts (e.g., sodium chloride), and sugars (e.g., dextrose, mannitol, sucrose and lactose). Preferably the isotonicity agent is mannitol.

10 The term "antioxidant" refers to a compound that prevents oxygen or oxygen-derived free radicals from interacting with other substances. Antioxidants are among a number of excipients commonly added to pharmaceutical systems to enhance physical and chemical stability. Antioxidants are added to minimize or retard oxidative processes that occur with some drugs or excipients upon exposure to oxygen or in the presence of free radicals. These processes can often be catalyzed by light, temperature, hydrogen ion concentration, presence of trace metals or peroxides. Sulfites, bisulfites, thiourea, methionine, salts of ethylenediaminetetraacetic acid (EDTA), butylated hydroxytoluene (BHT), and butylated hydroxy anisole (BHA) are frequently used as antioxidants in drugs. Sodium EDTA has been found to enhance the activity of antioxidants by chelating metallic ions that would otherwise catalyze the oxidation reaction. Most preferred antioxidant is methionine.

20 The term "bacteriostatic" refers to a compound or compositions added to a formulation to act as an anti-bacterial agent. A preserved interferon-containing formulation of the present invention preferably meets statutory or regulatory guidelines for preservative effectiveness to be a commercially viable multi-use product. Examples of bacteriostatics include phenol, *m*-cresol, *p*-cresol, *o*-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal. Preferably the bacteriostatic agent is benzyl alcohol.

30 In a preferred embodiment, the invention provides a stabilized liquid pharmaceutical composition comprising an interferon (IFN) or an isoform, muteln, fused protein, functional derivative, active fraction or salt thereof, wherein said formulation is

In a preferred embodiment, the invention provides a container according to the invention wherein said container is a pre-filled syringe for mono-dose administration.

In another embodiment, the invention provides a kit for multi-dose administration of a pharmaceutical composition according to the invention, wherein the kit comprises a first container filled with a pharmaceutical composition according to the invention and a second cartridge filled with a solution of the bacteriostatic agent.

Preferably the concentration of IFN-beta in the formulation is at or about 10 µg/ml to at or about 800 µg/ml, more preferably at or about 20 µg/ml to at or about 500 µg/ml, more particularly preferably at or about 30 to at or about 300, most preferably at or about 44, 88 or 264 µg/ml.

Preferably the formulations of the present invention have pH between about 3.0 and at or about 4.5, more preferably at or about 3.8. A preferred buffer is acetate, with preferred counterions being sodium or potassium ions. Acetate saline buffers are well known in the art. Buffer concentrations in total solution can vary between at or about 5 mM, 9.5 mM, 10 mM, 50 mM, 100 mM, 150 mM, 200 mM, 250 mM, and 500 mM. Preferably the buffer concentration is at or about 10mM. Particularly preferred is a buffer 50 mM in acetate ions with a pH of 3.8.

Preferably in the composition of the invention the antioxidant, for example methionine, is present at a concentration of at or about 0.01 to at or about 5 mg/ml, more preferably at or about 0.05 to at or about 0.3 mg/ml, most preferably at or about 0.1 mg/ml.

Preferably the concentration of the isotonicity agent (for example mannitol) in liquid formulations is at or about 0.5 mg/ml to at or about 500 mg/ml, more preferably at or about 1 mg/ml to at or about 250 mg/ml, more particularly preferably at or about 10 mg/ml to at or about 100 mg/ml, most preferably at or about 50 mg/ml.

In a further preferred embodiment, the invention provides a composition according to the invention wherein the isotonicity agent is mannitol, the antioxidant is methionine and the interferon is interferon beta.

equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

#### DESCRIPTION OF THE FIGURES

Figure 1: it shows the aggregation kinetic of interferon beta-1a 0.116 mg/mL (5.16  $\mu$ M) in PEG/PBS, after incubation at 62 $\pm$ 2  $^{\circ}$ C 10 min., in the presence of different concentrations of HPBCD: 1.19 mg/mL (150-fold molar excess with respect to molar amount of interferon beta-1a), 3.97 mg/mL (500-fold molar excess with respect to molar amount of interferon beta-1a), 5.56 mg/mL (700-fold molar excess with respect to molar amount of Interferon beta-1a) and 7.94 mg/mL (1000-fold molar excess with respect to molar amount of interferon beta-1a). In the Y axis is reported the optical density measured at 360 nm which is directly proportional to turbidimetry (*Cancellieri et al., BIOPOLYMERS, VOL 13, 735-743, 1974*).

Figure 2: it shows the effect of 10,000, 20,000 and 40,000 molar excess of mannitol on the aggregation of 0.116 mg/mL (5.1 $\mu$ M) interferon beta-1a in PBS/PEG10000 (after incubation at 62 $\pm$ 3  $^{\circ}$ C 10 min).

Figure 3: it shows aggregation kinetic of Interferon beta-1a In the presence of different concentrations of L-methionine: 0.077 mg/mL (100-fold molar excess with respect to molar amount of Interferon beta-1a), 0.158 mg/mL (205-fold molar excess with respect to molar amount of Interferon beta-1a), 0.308 mg/mL (400-fold molar excess with respect to molar amount of interferon beta-1a), 0.769 mg/mL (1000-fold molar excess with respect to molar amount of Interferon beta-1a) and 7.69 mg/mL (10000-fold molar excess with respect to molar amount of interferon beta-1a).

Figure 4: it shows the aggregation kinetic of interferon beta-1a alone and in the presence of methionine 400-fold molar excess with respect to molar amount of interferon beta-1a (0.308 mg/mL) and/or HPBCD 700-fold molar excess with respect to molar amount of interferon beta-1a (5.56 mg/mL)

Figure 5: it shows the effect of L-ascorbate 50-fold molar excess with respect to molar amount of interferon beta-1a (0.045 mg/mL), 150-fold molar excess with respect to molar amount of interferon beta-1a (0.136 mg/mL), 500-fold molar excess with respect to molar amount of Interferon beta-1a (0.453 mg/mL) and 10000-fold molar excess with respect to molar amount of Interferon beta-1a (9.07 mg/mL) on the aggregation of interferon beta-1a 0.116 mg/mL (5.16  $\mu$ M) in PEG/PBS, after incubation at 62  $\pm$  2  $^{\circ}$ C, 10 minutes.

- 10 Figure 6: It reports the interferon beta-1a bulk (about 44  $\mu$ g/mL) thermal denaturation (i.e. effect of temperature on CD signal at 222 nm) in the upper part of the figure and the relative CD spectrum before (solid line) and after (dash line) melting transition in the lower part of the figure. Hereinafter the respective CDNN (Circular Dichroism Neural Network) deconvolutions (average of four analyses) are reported.
- 15 N.B.: The melting transition curve or thermal denaturation represents the effect of temperature on CD signal at 222 nm. All CD graphs have in Y-axis the molar ellipticity as  $\text{deg M}^{-1} \text{cm}^{-1}$  (Y axis)
- In the table the alpha helix residual in the range of 200-260 nm has been considered for the comparison of IFN conformational stability in the pre/post melting CDNN deconvolutions (average of four analyses).
- 20

Figure 7: the thermal denaturation curve is reported for a solution containing interferon beta-1a about 44  $\mu$ g/mL and HPBCD 2.11 mg/mL (700-fold molar excess with respect to molar amount of Interferon beta-1a), i.e. the effect of temperature on CD signal at 222 nm, expressed as molar ellipticity,  $\text{deg M}^{-1} \text{cm}^{-1}$ . In the upper part of the figure (solid line), compared with the protein alone (dash line) and the relative CD spectrum before (solid line) and after (dash line) melting transition in the lower part of the figure. Hereinafter the respective CDNN (Circular Dichroism Neural Network) deconvolutions (average of three analyses) are reported.

- 30 Figure 8: it shows the thermal denaturation of interferon beta-1a alone (dash curve) and in the presence of Na L-ascorbate 500-fold molar excess with respect to molar amount of interferon beta-1a (solid curve).

Figure 9: it shows [interferon beta-1a/ascorbate 500x] CD spectrum before (solid curve) and after (dash curve) melting transition and respective CDNN deconvolutions (see above).

5. Figure 10: it shows the aggregation kinetic of interferon beta-1a 0.116 mg/mL (5.16  $\mu$ M) in PEG/PBS (final pH = 4.7) after incubation at 62 $\pm$ 2 °C 10 min. and the effect of a 700x M excess of HPBCD (5.56 mg/mL). In the Y axis is reported the optical density measured at 360 nm which is directly proportional to turbidimetry.
10. Figure 11: it shows the aggregation kinetic of interferon beta-1a 0.116 mg/mL (5.16  $\mu$ M) in PEG/PBS (final pH = 5.1) after incubation at 62 $\pm$ 2 °C 10 min. and the effect of a 700x M excess of HPBCD (5.56 mg/mL) and 500x M excess of RMBCD (3.38 mg/mL).
15. Figure 12: it shows the aggregation kinetic of interferon beta-1a 0.116 mg/mL (5.16  $\mu$ M) in PEG/PBS (final pH = 5.7) after incubation at 62 $\pm$ 2 °C 10 min. and the effect of a 700x M excess of HPBCD (5.56 mg/mL).

#### EXAMPLES

20. The following abbreviations refer respectively to the definitions below:  
 cm (centimeter), mg (milligram),  $\mu$ g (microgram), min (minute), mM (millimolar), mL (milliliter), nm (nanometer), BHT (butylated hydroxytoluene), CD (Circular dichroism), EDTA (ethylenediaminetetraacetic acid), HPBCD (Hydroxypropyl-beta-cyclodextrin), HPLC (High Performance Liquid Chromatography), IFN (interferon), IM (Intramuscular), OD (Optical Density), PBS (Phosphate Buffered Saline), PEG (Polyethylene glycol), RMBCD (randomly substituted methyl-beta-cyclodextrin), SC (subcutaneous), TFA (trifluoroacetic acid), TRIS (2-amino-2-hydroxymethyl-1,3-propanediol, UV (Ultraviolet), WFI (Water For Injection).

#### 30. Methods

##### Turbidimetry Measurements

Protein aggregation was monitored for 30 min at 360 nm using an UV-visible spectrophotometer system (Perkin Elmer Lambda 40).

The operative conditions used were:

- chromatographic column: TSK G2000 SW<sub>XL</sub> (7.8 mm ID x 30 cm, 5  $\mu$ , 125 Å);
- injection volume: 100  $\mu$ L;
- 5 • column temperature: room temperature;
- sample temperature: room temperature;
- flow rate: 0.5 mL/min;
- mobile phase: 70 % v/v purified water (MILLIQ-Millipore)-30 % v/v acetonitrile-0,2 % v/v TFA;
- 10 • run time: 27 min;
- equilibration time: 3 min;
- wavelength: 214 nm;
- A calibration curve, ranged from 25 $\mu$ g up to 100 $\mu$ g, was employed to quantify interferon beta-1a assay.

#### 15 Materials

- Interferon beta-1a bulk (Serono S.A., batch G4D024)
- PEG 10000 (polyethylenglicole)
- Lutrol F68 (polyoxyethilene-polyoxypropilene block copolymer)
- L-Methionine
- 20 • D-Mannitol
- L-Ascorbic acid
- Phosphate Buffer Saline buffer pH 7.4  $\pm$  0.1 (composition: KH<sub>2</sub>PO<sub>4</sub> 0.19 g/L, Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 2.38 g/L, NaCl 8 g/L)
- Hydroxypropyl-beta-cyclodextrin

25

#### Equipment

- HPLC systems (Waters and PE) equipped with TSK Column.G2000.
- UV-visible spectrophotometer system (Perkin Elmer Lambda 40)
- Jasco J810 spectropolarimeter equipped with a Peltier temperature controller
- 30 • Osmometer (OSMOMAT 030D, Gonotech)
- PH-conductivity Meter MPC 227-Mettler Toledo
- Analytical balance AG245 and AG 285 (Mettler Toledo)
- Calibrated pipettes (Gilson)

- Magnetic stirrer hot plate (Stuart Scientific)
- Ultrasonic bath, Falc
- Thermometers

## 5 Results And Discussion

### Turbidimetry assay

The effect on interferon beta-1a aggregation of HPBCD, mannitol and L-methionine, detected by turbidity method, is reported below. Sodium ascorbate salt is also included as example of excipient having an aggregation-enhancement effect.

- 10 Figure 1 shows the aggregation kinetic of interferon beta-1a in the presence of different concentrations of HPBCD: 1.19 mg/mL (150-fold molar excess with respect to molar amount of interferon beta-1a), 3.97 mg/mL (500-fold molar excess), 5.56 mg/mL (700-fold molar excess) and 7.94 mg/mL (1,000-molar excess). It can be noticed that in the concentration range investigated this excipient does not avoid completely interferon
- 15 beta-1a destabilization and that intermediate molar ratios show the best inhibitory effect.

700-fold molar excess was chosen as reference concentration for Interferon beta-1a formulation preparation and further physico-chemical characterization (e.g. circular dichroism). The concentration of cyclodextrin is better expressed as molar ratio

20 vs Interferon beta-1a (-fold molar excess) as the concentration varies depending on the interferon beta-1a quantity used in the preparation and can be calculated accordingly.

The influence of mannitol on interferon beta-1a aggregation was then monitored but no significant effect was found even at 40,000-fold molar excess (corresponding to 37,35 mg/mL) under the conditions used (62°C in PEG/PBS), as shown in Figure 2.

- 25 However mannitol was added to Interferon beta-1a liquid formulations in order to reach isotonicity necessary for parenteral administration.

Finally, L-Methionine was tested in a turbidimetry experiment. Figure 3 shows Aggregation kinetic of interferon beta-1a (0.116 mg/mL, in PEG/PBS, after incubation at 62 +/- 2 °C, 10') in the presence of different concentrations of L-methionine

- 30 It can be noticed that L-methionine has a less inhibitory effect on protein aggregation than HPBCD: even at the maximum investigated concentration it does not avoid completely interferon beta-1a destabilization and the curve reaches a "plateau" similar to interferon beta-1a control.

In Figure 4, the aggregation kinetic of interferon beta-1a alone and in the presence of methionine 400-fold molar excess (0.308 mg/mL) and/or HPBCD 700-fold molar excess (5.56 mg/mL) is reported.

This experiment was carried out in order to assess eventually a synergistic effect or interference of these two excipients. It's clear that methionine exerts no protective effect against protein aggregation in addition to the activity of the cyclodextrin.

Following above considerations it was confirmed that the HPBCD was playing a major role in the stabilization of interferon beta-1a toward aggregation and the interaction between the protein and the cyclodextrin was further investigated by circular dichroism.

As example of excipient having "negative effect" on protein aggregation in Figure 5 the turbidity kinetic of interferon beta-1a in the presence of different concentrations of ascorbate salt is reported.

The effect of L-ascorbate 50-fold molar excess (0.045 mg/mL), 150-fold molar excess (0.136 mg/mL), 500-fold molar excess (0.453 mg/mL) and 10,000-fold molar excess (9.07 mg/mL) on the aggregation of interferon beta-1a 0.116 mg/mL (5.16  $\mu$ M) in PEG/PBS, after incubation at 62  $\pm$  2°C, 10 min. is shown in Figure 5.

It is interesting to notice that the effect on the protein aggregation varies in a concentration-dependent manner: at high concentration negative charges seem to display a destabilizing effect, while at lower molar ratios this excipient seems to have an inhibitory influence.

However it was possible to identify a concentration (0.453 mg/mL corresponding to excipient/interferon beta-1a molar ratio equal to 500) that seems to hamper protein aggregation.

The ascorbate was then chosen as possible excipient for interferon beta-1a formulations, with the purpose of combining its antioxidant action with a specific anti-aggregation effect.

### *Circular Dichroism*

In Figure 6 thermally induced unfolding, between 25°C and 85°C, and far-UV spectra of interferon beta-1a bulk sample (about 44  $\mu$ g/mL) are reported. It can be noticed that the estimated  $T_m$  value is 64.97  $\pm$  0.31°C, while spectrum deconvolution

presence of possibly changed conformation, as suggested also by CD results. This was the reason why the stability of the formulation containing ascorbate was not performed at higher temperatures (e.g. 50°C).

- 5 In the latter case it was important to verify the initial promising result obtained by turbidimetry analysis with alternative methods such as circular dichroism that is able to monitor the effect of a known excipient on the protein conformation stability.

#### *Turbidimetry assay at higher pH*

- 10 The effect on interferon beta-1a aggregation of HPBCD in a wider pH range of the liquid formulation (i.e. pH about 3.0 to about 4.0) was investigated.

- The method consisted in the previously described turbidimetric assay: Interferon beta-1a bulk was diluted 1:2 with a solution of PEG 10,000 30 mg/mL in PBS (0.2 µm Ø filtered and properly basified by adding a small volume of NaOH 1N) and then incubated in a thermostatic water bath at  $T = 62 \pm 2^\circ\text{C}$  for 10 min. Protein aggregation was monitored for 30 min at 360 nm using an UV-visible spectrophotometer system (Perkin Elmer Lambda 40). Each turbidity analysis was repeated in duplicate and the optical density (OD)<sub>360nm</sub> versus time average curve is reported. Aggregation of the protein alone was compared to Interferon beta-1a in the presence of cyclodextrin.

- 20 The dilution of acetate buffer 50 mM (i.e. IFN bulk medium) 1:1 by PBS leads to a final solution pH of 4.4. The aim was therefore to investigate protein aggregation for higher final pH.

Figure 10 shows the aggregation kinetic of interferon beta-1a in the absence and in the presence of a 700x M excess of HPBCD (5.56 mg/mL), with a pH of the analysed solution equal to 4.7 at room temperature.

- 25 It can be noticed that an increment of pH enhances the extent of IFN aggregation, but the presence of cyclodextrin still partially inhibits protein destabilization. The relative percentage OD (i.e. percentage ratio between OD<sub>360 nm</sub> after 30 min in the presence and in the absence of the excipient) calculated for this experiment is 86 %, not so far from what observed in the usual operative conditions at lower pH (52.7 %).

30 The study was extended to a higher pH, i.e. 5.1, as shown in figure 11: the effect of HPBCD at a 700 M excess (5.56 mg/mL) and of RMBCD at a 500 M excess (3.38 mg/mL) on IFN aggregation was investigated. It can be noticed a more marked

protein destabilization due to the increase of pH and the almost total absence of a kinetic trend (i.e. plateau region at the beginning of the analysis). The interesting finding is that IFN aggregation is still partially inhibited by cyclodextrins, with a relative percentage OD equal to 69.7 % in the case of HPBCD (no advantage can be observed by the use of the methyl-derivative in this case).

A third pH value was investigated. Figure 12 shows IFN aggregation kinetic in PEG/PBS at pH 5.7 and the effect of addition of a 700x molar excess of HPBCD. The excipient does not avoid protein destabilization, but significantly reduces its extent with respect to IFN control (relative percentage OD 62.6 %).

The above considerations indicate that the use of HPBCD as stabilizing excipient could be extended to liquid formulation at pH higher than the protein bulk characteristic value (i.e. pH 3.8  $\pm$  0.5).

### Conclusions

- Some Interferon beta-1a liquid formulations were prepared and kept in stability at room temperature (25°C) and in accelerated conditions (50°C).
  - The most stable formulation contains L-methionine, HPBCD and mannitol. SE-HPLC results show a monomer content above 90% after 1 week at 50 °C or 1 month at 25°C.
  - The positive result of HPBCD was anticipated and confirmed by turbidimetry measurement that showed a concentration dependent-inhibition effect of this excipient, (and partially also of L-methionine), toward interferon beta-1a aggregation. Furthermore CD analysis showed that in the presence of HP-beta-cyclodextrin there is clearly a smaller interferon beta-1a  $\alpha$ -helix loss after melting transition.
  - The Interferon beta-1a bulk, kept in the same storage conditions, displays a different stability profile; the monomer content decreased down to 83% after 1 week at 50°C.
- Of note at 25°C the monomer content after 1 month is still equal to 97 %, which is surprisingly high. This result could be explained by the fact that the bulk contains acetate buffer at pH 3.8. This condition has itself a certain degree of stabilizing effect for interferon beta-1a.

REFERENCES

1. Arakawa, Prestrelski, Kenney and Carpenter (2001), "Factors affecting short-term and long-term stabilities of proteins", *Adv. Drug Deliv. Rev.* 46(1-3):307-326;
- 5 2. Brewster et al., 1991, *Pharmaceutical research*, New York, 8(6), 792-795;
3. Cancellieri et al., *Biopolymers*, VOL 13, 735-743, 1974;
4. Clegg and Bryant, *Exp. Opin. Pharmacother* 2001; 2(4): 623-639;
5. Derynk R. et al., *Nature* 1980; 285, 542-547 ;
6. Familletti, P. C., Rubinstein, S., and Pestka, S. 1981 "A Convenient and Rapid  
10 Cytopathic Effect Inhibition Assay for Interferon," In *Methods in Enzymology*, Vol. 78 (S. Pestka, ed.), Academic Press, New York, 387-394;
7. Hultgren C, Milich DR, Welland O, Sallberg M. (1998). The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. *J Gen Virol* 1998; 79 :2381-2391;
- 15 8. Irie et al., 1999, *Adv. Drug Deliv. Rev.* , Vol 36, 101-123;
9. McCormick JB, Kling IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH, Belmont-Williams R. Lassa fever. Effective therapy with ribavirin. *N Engl J Med.* 1986 Jan 2; 314(1):20-6;
10. Mark D.F. et al., *Proc. Natl. Acad. Sci. U.S.A.*, 81 (18) 5662-5666 (1984);
- 20 11. Pagington, *Chemistry in Britain*, pp. 455-458 (1987);
12. Pestka, S. (1986) "Interferon Standards and General Abbreviations," in *Methods in Enzymology* (S. Pestka, ed.), Academic Press, New York 119, 14-23;
13. Piha et al, *International Journal of Pharmaceutics*, 29, 73-82 (1986);
14. Piha et al, in *Controlled Drug Delivery*, ed. S. D. Bruck, Vol. I, CRC Press, Boca  
25 Raton, Fla., pp. 125-148 (1983);
15. Rubinstein, S., Familletti, P.C., and Pestka, S. Convenient Assay for Interferons. *J. Virol* 1981; 37, 755-758 ;
16. Shepard H. M. et al., *Nature* 1981; 294, 563-565;
17. T. Irie et al., *Cyclodextrins in peptide and protein delivery*, *Adv. Drug Deliv. Rev.* ,  
30 Vol 36, 101-123 (1999);
18. Study Group. *The Lancet* 1998; 352, 1498-1504;
19. Uekama et al, in *CRC Critical Reviews in Therapeutic Drug Carrier Systems*, Vol. 3 (1), 1-40 (1987);

20. Uekama, in *Topics in Pharmaceutical Sciences 1987*, eds. Breimer and  
Speiser, Elsevier Science Publishers B.V. (Biomedical Division), 181-194  
(1987);
21. Wang et al., *Int. J. Pharm.*, 185:129-188 ;
- 5 22. Wang et al., *J. Parenteral Sci. Tech.*, 1998, 42:S3-S26;
23. WO 03 00/2152;
24. WO 99/55377;
25. WO 90/03784;
26. US 6,582,728;
- 10 27. US 6,013,253;
28. US 5,997,856;
29. US 5,541,293;
30. US 5,116,943;
31. US 5,017,691;
- 15 32. US 4,965,195;
33. US 4,959,314;
34. US 4,904,584;
35. US 4,897,471;
36. US 4,879,111;
- 20 37. US 4,737,462;
38. US 4,695,623;
39. US 4,588,585.

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,846,427

Page 1 of 4

APPLICATION NO.: 10/582,027

DATED : December 7, 2010

INVENTOR : Maria Dorly Del Curto

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1.

Line 60, "Burkit's lymphoma" should read --Burkitt's lymphoma--.

Column 2.

Line 10, "There is a duster" should read --There is a cluster--.

Line 19, "agents can are sometimes" should read --agents are sometimes--.

Lines 29-30, "166 amino acid" should read --166 amino acids--.

Column 3.

Line 4, "cydodextrins are" should read --cyclodextrins are--.

Line 18, "(2,6-dimethyl)-beta-cyclodextrin" should read

--(2,6-di-O-methyl)-beta-cyclodextrin--.

Line 31, "has bee" should read --has been--.

Line 32, "T. Ide" should read --T. Irie--.

Line 40, "stabilization if" should read --stabilization of--.

Column 4.

Line 2, "hum an serum" should read --human serum--.

Line 58, "polynucleotide's" should read --polynucleotides--.

Column 5.

Line 7, "amino adds" should read --amino acids--.

Line 14, "amino add" should read --amino acid--.

MAILING ADDRESS OF SENDER:

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UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,846,427

Page 2 of 4

APPLICATION NO.: 10/582,027

DATED : December 7, 2010

INVENTOR : Maria Dorly Del Curto

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 7.

Line 65, "sulfuric add, and salts with organic adds" should read

--sulfuric acid, and salts with organic acids--.

Line 66, "oxalic add" should read --oxalic acid--.

Column 8.

Line 7, "so called" should read --so-called--.

Line 23, "amino add" should read --amino acid--.

Line 32, "tripe tide" should read --tripeptide--.

Line 43, "an IgG<sub>2</sub>, molecule." should read --an Ig molecule.--.

Lines 64-65, "80 000 IU/kg and 200 000" should read --80,000 IU/kg and 200,000--.

Column 9.

Line 9, "one, twice" should read --once, twice--.

Line 15, "IFN- $\beta$  In the" should read --IFN- $\beta$  in the--.

Column 10.

Lines 42-43, "hydroxypropyl-gaamma-cyclodextrin" should read

--hydroxypropyl-gamma-cyclodextrin--.

Line 45, "or GIBCD)" should read --or G<sub>1</sub>BCD)--.

Line 50, "maltotriosylgamma-cyclodextrin" should read

--maltotriosyl-gamma-cyclodextrin--.

Lines 52-53, "maltosyl-beta-cydodextrin/dimaltosyl-beta-dodextrin" should read

--maltosyl-beta-cyclodextrin/dimaltosyl-beta-cyclodextrin--.

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PATENT NO. : 7,846,427

Page 3 of 4

APPLICATION NO.: 10/582,027

DATED : December 7, 2010

INVENTOR : Maria Dorly Del Curto

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 11.

Line 21, "bacterostatic" should read --bacteriostatic--.

Line 26, "product Examples" should read --product. Examples--.

Column 13.

Line 4, "IFN beta" should read --IFN-beta--.

Line 14, "in the are buffer" should read --in the art. Buffer--.

Column 15.

Line 40, "the invention include" should read --the invention includes--.

Column 16.

Line 53, "amount if" should read --amount of--.

Line 65, "amount if" should read --amount of--.

Column 17.

Line 7, "malting" should read --melting--.

Line 46, "and 5(0x M" should read --and 500x M--.

Column 18.

Line 48, "5 p" should read --5  $\mu$ --.

Line 59, "up to 10  $\mu$ g" should read -- up to 100 $\mu$ g--.

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CERTIFICATE OF CORRECTION

PATENT NO. : 7,846,427

Page 4 of 4

APPLICATION NO.: 10/582,027

DATED : December 7, 2010

INVENTOR : Maria Dorly Del Curto

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 19.

Line 5, "Hydroxypropyl-betacyclodextrin" should read

--Hydroxypropyl-beta-cyclodextrin--.

Line 56, "isotonidity" should read --isotonicity--.

Column 20.

Line 26, "manner at" should read --manner: at--.

Line 31, "excipient interferon" should read --excipient/interferon--.

Column 23.

Line 12, "about 4-0)" should read --about 4.0)--.

Line 14, "diluted 12" should read --diluted 1:2--.

Line 21, "(OD)<sub>380 min</sub>" should read --(OD)<sub>360nm</sub>--.

Line 36, "OD 96 nm" should read --OD<sub>360 nm</sub>--.

Column 24.

Line 11, "a-helix" should read -- $\alpha$ -helix--.

Column 25.

Line 8, "8. T. Irie" should read --8. Irie--.

Line 59, "140 (1987)" should read --1-40 (1987)--.

Line 64, "22. Wang et a." should read --22. Wang et al.--.

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